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09/781 893

L2: Entry 1 of 17

File: USPT

Jul 2, 2002

DOCUMENT-IDENTIFIER: US 6413949 B1 .. /

TITLE: Prodrugs with enhanced penetration into cells

Brief Summary Text (9):

In other examples of phospholipid prodrugs, formulation of the prodrugs into liposomes or other micellar structures is the feature that enables their preferential uptake, for instance by macrophages or by liver cells as in the case of the phospholipid conjugates of antiviral drugs disclosed in WO 90/00555 and WO 93/00910.

Brief Summary Text (10):

Generally, viral infection is not associated with supranormal phospholipase activity and antiviral phospholipid conjugates do not teach or suggest activation of the drug preferentially in the diseased cells, or in the infected cells as in the case of the phospholipid conjugates of antiviral nucleotides and anti-sense oligonucleotides, such as those disclosed in WO 90/00555, in WO 90/10448 and in NTIS Technical Notes, no. 9, page 630, Springfield, Va., US, 1984.

Detailed Description Text (7):

In currently preferred embodiments, the enzymes that activate the prodrugs are intracellular lipases. In most preferred embodiments the covalent bond of the prodrug is scission sensitive to phospholipases, a non limiting example of which are the phospholipases A2.

Detailed Description Text (8):

Distinction among the various phospholipases is based in part on their substrate specificity as well as their tissue localization, regulation and physicochemical attributes. The different specificities of these classes of phospholipases can serve as the basis of designing prodrugs which undergo specific activation, as suitable for the pathology to be treated.

Detailed Description Text (9):

The cleavage sites of the various phospholipases are herein depicted schematically in the following scheme. ##STR1##

Detailed Description Text (10):

Prodrugs designed as substrates for phospholipase C (PLC) will be much more useful for treatment of chronic excitatory disorders such as epilepsy. In this type of disorder PLC is involved in the earliest events of hyperactivation (preceding the physiological attack), while PLA.sub.2 activation coincides with epileptic seizures.

Detailed Description Text (11):

Prodrug activation by PLC could be most preferred for targeting of antiepileptic drugs. Whereas prodrug activation by Phospholipase D (PLD) could be appropriate for targeting of antitumor drugs. In such prodrugs the P--O bond constituting the bond between the drug and the phospholipid would be scission-sensitive to enzyme PLD, thus releasing the antitumor agents intracellularly, and accumulating these inhibitors in cells having a supranormal level of PLD.

Detailed Description Text (12):

Phospholipases A.sub.2 are a family of esterases that hydrolyze the sn-2 ester bonds in phosphoglyceride molecules releasing a free fatty acid and a lysophospholipid. Classification of the members of this family of enzymes is based on certain

structural features and/or their localization in different cells and tissues. In principle, these enzymes are more active on aggregated phospholipid substrates compared with monomeric soluble substrates.

Detailed Description Text (13):

Phospholipid conjugates of drugs that will be cleaved by Phospholipases A.sub.2 have previously been disclosed either a) to enhance penetration into cells; b) to enable formulation of drugs in liposomes; or c) as a form of "enterocoating" that prevents exposure of the gastric mucosa to the drug.

Detailed Description Text (14):

None of the previously disclosed uses of phospholipid-drug conjugates is an essential feature of the present methods of using these prodrugs, inasmuch as a) the present invention is effective even with drugs that are already capable of penetrating cells, as in the example of antiepileptic drugs; b) it is not desirable according to the current invention to formulate the prodrugs into liposomes since this achieves preferential distribution to specific organs (e.g., the liver) or to specific cell types (e.g., macrophages) rather than to diseased cells within an organ or cell population; c) the prodrugs according to the present invention are intended for parenteral administration in order to prevent their premature digestion by phospholipases in the digestive tract.

Detailed Description Text (16):

According to one preferred embodiment of the present invention, protease inhibitors are provided which comprise a peptide or peptide analog which is a potent protease inhibitor, covalently bound to a phospholipid. These prodrugs are cell permeable molecules which are scission sensitive to abnormally hyperactivated phospholipases. Preferred protease inhibitors may include peptides, peptide analogs, or peptidomimetics.

Detailed Description Text (20):

Prodrugs activated by phospholipases

Detailed Description Text (30):

The choice of the preferred alcohol that is appropriate for any given prodrug is dependent on the intended therapeutic use of the conjugate. Thus alcohols below C.sub.10 exhibit very low substrate specificity, whereas alcohols above C.sub.12 or C.sub.14 are very good substrates for the phospholipases and will therefore be readily activated. Regulated activation will best be achieved by the intermediate length alcohols such as C.sub.2-10, and these will be preferred for the treatment of persistent or chronic disease states or disorders.

Detailed Description Text (60):

A solution of the product of part (a) (1.2 mmol) in a mixture of ether (196 ml) and methanol (12 ml) was stirred vigorously in presence of (HOCH.sub.2).sub.3 C--NH.sub.2.HCl (50 ml of 0.1M, pH 8.7) containing CaCl.sub.2 (0.72 mM) and 5 mg of crude rattlesnake venom (*Crotalus adamanteus*) as a source of phospholipase A.sub.2, at 37.degree. C. for 3 hours. The reaction was monitored by TLC (70:25:4 by volume chloroform/methanol/water). After completion of reaction, the organic layer was separated, and the aqueous layer was washed with ether and then lyophilized. The residue was extracted with 2:1 by volume chloroform/methanol and centrifuged. On evaporation of the clear supernatant, the title product was obtained in 90% yield. Thin layer chromatography using 70:25:4 by volume chloroform/methanol/water showed that it was free from starting material and heptanoic acid. Any fatty acid in the product can however be removed by crystallization from ethanol-ether.

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White rats were sacrificed by cervical dislocation and their hearts were rapidly removed and reperfused at 60 mmHg with modified Krebs-Henseleit buffer utilizing a Langendorff perfused heart model. Hearts were perfused for 10-min. preequilibration interval and were subsequently rendered either global ischemic (zero flow) or continuously perfused for the indicated time. Perfusion was terminated by rapid excision of ventricular tissue and directly submersion into cold homogenization buffer (10 mM imidazole, 10 mM KCl, 0.25 M sucrose [grade 1], pH 7.8) Both the activation of phospholipase A2 and its reversibility during reperfusion were

temporally correlated to alterations in myocytic anaerobic metabolism and electron microscopic analyses.

Other Reference Publication (7):

Natarajan et al. "Activation of endothelial cell phospholipase D by hydrogen peroxide and fatty acid hydroperoxide." J. Biol. Chem 268(2):930-7, 1989.

Other Reference Publication (8):

Coorssen et al., "GTP.gamma.S and phorbol ester act synergistically to stimulate both calcium independent secretion and phospholipase D activity in permeabilized human platelets. Inhibition by BAPTA and analogs" FEBS Lett. 316(2):170-4, 1991.

CLAIMS:

7. The prodrug according to claim 6, wherein said lipase is phospholipase.
8. The prodrug according to claim 3, wherein said covalent bond is scission-sensitive to phospholipases.
9. The prodrug according to claim 8, wherein said phospholipase is selected from the group consisting of phospholipase A.sub.2 and phospholipase C.

**WEST**[Generate Collection](#)[Print](#)**Search Results - Record(s) 1 through 17 of 17 returned.**☐ 1. Document ID: US 6413949 B1

L2: Entry 1 of 17

File: USPT

Jul 2, 2002

US-PAT-NO: 6413949

DOCUMENT-IDENTIFIER: US 6413949 B1

TITLE: Prodrugs with enhanced penetration into cells

DATE-ISSUED: July 2, 2002

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kozak; Alexander	Rehovot			IL

US-CL-CURRENT: [514/143](#); [514/148](#), [514/546](#), [514/547](#), [514/549](#), [514/552](#), [554/80](#)

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC
Draw Desc	Image										

☐ 2. Document ID: US 6355629 B2

L2: Entry 2 of 17

File: USPT

Mar 12, 2002

US-PAT-NO: 6355629

DOCUMENT-IDENTIFIER: US 6355629 B2

TITLE: Prodrugs with enhanced penetration into cells

DATE-ISSUED: March 12, 2002

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kozak; Alexander	Rehovot			IL

US-CL-CURRENT: [514/143](#); [514/183](#), [514/533](#), [514/534](#), [554/103](#), [554/104](#), [554/107](#), [554/79](#)

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC
Draw Desc	Image										

☐ 3. Document ID: US 6229002 B1

L2: Entry 3 of 17

File: USPT

May 8, 2001

US-PAT-NO: 6229002

DOCUMENT-IDENTIFIER: US 6229002 B1

TITLE: Platelet derived growth factor (PDGF) nucleic acid ligand complexes

DATE-ISSUED: May 8, 2001

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Janjic; Nebojsa	Boulder	CO		
Gold; Larry	Boulder	CO		

US-CL-CURRENT: 536/23.1; 435/6, 435/91.2

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KMIC
Draw	Desc	Image									

☐ 4. Document ID: US 6180114 B1

L2: Entry 4 of 17

File: USPT

Jan 30, 2001

US-PAT-NO: 6180114

DOCUMENT-IDENTIFIER: US 6180114 B1

TITLE: Therapeutic delivery using compounds self-assembled into high axial ratio microstructures

DATE-ISSUED: January 30, 2001

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Yager; Paul	Seattle	WA		
Gelb; Michael H.	Seattle	WA		
Lukyanov; Anatoly N.	Seattle	WA		
Goldstein; Alex S.	Seattle	WA		
Disis; Mary L.	Renton	WA		

US-CL-CURRENT: 424/400; 424/409, 424/450, 514/44

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KMIC
Draw	Desc	Image								

☐ 5. Document ID: US 6166089 A

L2: Entry 5 of 17

File: USPT

Dec 26, 2000

US-PAT-NO: 6166089

DOCUMENT-IDENTIFIER: US 6166089 A

TITLE: Prodrugs with enhanced penetration into cells

DATE-ISSUED: December 26, 2000

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kozak; Alexander	Rehovat			IL

US-CL-CURRENT: 514/642; 514/144, 514/557

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KMOC
Draw Desc	Image									

☐ 6. Document ID: US 6077837 A

L2: Entry 6 of 17

File: USPT

Jun 20, 2000

US-PAT-NO: 6077837

DOCUMENT-IDENTIFIER: US 6077837 A

TITLE: Prodrugs with enhanced penetration into cells

DATE-ISSUED: June 20, 2000

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kozak; Alexander	Rehovat			IL

US-CL-CURRENT: 514/77; 514/114, 514/117, 514/143, 514/144, 514/76, 514/826, 554/78, 554/79, 554/80

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KMOC
Draw Desc	Image									

☐ 7. Document ID: US 5985854 A

L2: Entry 7 of 17

File: USPT

Nov 16, 1999

US-PAT-NO: 5985854

DOCUMENT-IDENTIFIER: US 5985854 A

TITLE: Prodrugs with enhanced penetration into cells

DATE-ISSUED: November 16, 1999

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kozak; Alexander	Rehovat			IL

US-CL-CURRENT: 514/75; 514/529, 514/557, 514/77, 514/78

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KMOC
Draw Desc	Image									

☐ 8. Document ID: US 5851536 A

L2: Entry 8 of 17

File: USPT

Dec 22, 1998

US-PAT-NO: 5851536  
DOCUMENT-IDENTIFIER: US 5851536 A

TITLE: Therapeutic delivery using compounds self-assembled into high axial ratio microstructures

DATE-ISSUED: December 22, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Yager; Paul	Seattle	WA		
Gelb; Michael H.	Seattle	WA		
Carlson; Paul A.	Seattle	WA		
Lee; Kyujin C.	Seattle	WA		
Lukyanov; Anatoly N.	Seattle	WA		
Goldstein; Alex S.	Seattle	WA		

US-CL-CURRENT: 424/400; 424/450

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KMIC
Draw Desc	Image									

☐ 9. Document ID: US 5846743 A

L2: Entry 9 of 17

File: USPT

Dec 8, 1998

US-PAT-NO: 5846743  
DOCUMENT-IDENTIFIER: US 5846743 A

TITLE: Polyphosphoinositide binding peptides for intracellular drug delivery

DATE-ISSUED: December 8, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Janmey; Paul A.	Arlington	MA		
Cunningham; C. Casey	Wayland	MA		
Hartwig; John H.	Jamaica Plain	MA		
Stossel; Thomas P.	Belmont	MA		
Vegner; Roland	Riga			LV

US-CL-CURRENT: 435/7.8; 435/244, 435/4, 435/6, 435/7.21, 435/7.23, 514/15, 514/16, 530/328

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KMIC
Draw Desc	Image									

☐ 10. Document ID: US 5817638 A

L2: Entry 10 of 17

File: USPT

Oct 6, 1998

US-PAT-NO: 5817638

DOCUMENT-IDENTIFIER: US 5817638 A

TITLE: Antiviral liponucleosides: treatment of hepatitis B

DATE-ISSUED: October 6, 1998

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hostetler; Karl Y.	Del Mar	CA		

US-CL-CURRENT: 514/45; 424/450, 514/46, 514/47, 514/48, 514/49, 514/50, 514/51,  
514/894, 536/26.23, 536/26.26, 536/26.7, 536/26.8, 536/26.9, 536/27.14, 536/28.2

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KWIC
Draw Desc	Image									

☐ 11. Document ID: US 5783662 A

L2: Entry 11 of 17

File: USPT

Jul 21, 1998

US-PAT-NO: 5783662

DOCUMENT-IDENTIFIER: US 5783662 A

TITLE: Polyphosphoinositide binding peptides for intracellular drug delivery

DATE-ISSUED: July 21, 1998

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Janmey; Paul A.	Arlington	MA		
Cunningham; C. Casey	Wayland	MA		
Hartwig; John H.	Jamaica Plain	MA		
Stossel; Thomas P.	Belmont	MA		
Vegner; Roland	Riga			LV

US-CL-CURRENT: 530/328; 530/329, 530/330, 530/345

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KWIC
Draw Desc	Image									

☐ 12. Document ID: US 5753262 A

L2: Entry 12 of 17

File: USPT

May 19, 1998

US-PAT-NO: 5753262

DOCUMENT-IDENTIFIER: US 5753262 A

TITLE: Cationic lipid acid salt of 3beta[N- (N', N'-dimethylaminoethane) -  
carbamoyl]cholesterol and halogenated solvent-free preliposomal lyophilate thereof

DATE-ISSUED: May 19, 1998

## INVENTOR-INFORMATION:



NAME	CITY	STATE	ZIP CODE	COUNTRY
Wyse; Joseph W.	The Woodlands	TX		
Warner; Charles D.	The Woodlands	TX		

US-CL-CURRENT: 424/450; 435/458, 436/71, 552/545

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KWIC
Draw Desc	Image									

☐ 13. Document ID: US 5662932 A

L2: Entry 13 of 17

File: USPT

Sep 2, 1997

US-PAT-NO: 5662932

DOCUMENT-IDENTIFIER: US 5662932 A

TITLE: Solid fat nanoemulsions

DATE-ISSUED: September 2, 1997

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Amselem; Shimon	Rehovot			IL
Friedman; Doron	Carmei Yosef			IL

US-CL-CURRENT: 424/450; 424/45, 424/489, 424/490, 424/502, 428/402.2, 514/937

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KWIC
Draw Desc	Image									

☐ 14. Document ID: US 5576016 A

L2: Entry 14 of 17

File: USPT

Nov 19, 1996

US-PAT-NO: 5576016

DOCUMENT-IDENTIFIER: US 5576016 A

TITLE: Solid fat nanoemulsions as drug delivery vehicles

DATE-ISSUED: November 19, 1996

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Amselem; Shimon	Rehovot			IL
Friedman; Doron	Carmei Yosef			IL

US-CL-CURRENT: 424/450; 424/489, 424/490, 424/502, 428/402.2

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KWIC
Draw Desc	Image									

☐ 15. Document ID: US 5484911 A

L2: Entry 15 of 17

File: USPT

Jan 16, 1996

US-PAT-NO: 5484911

DOCUMENT-IDENTIFIER: US 5484911 A

TITLE: Nucleoside 5'-diphosphate conjugates of ether lipids

DATE-ISSUED: January 16, 1996

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hong; Chung I.	East Amherst	NY		
West; Charles R.	East Amherst	NY		
Chu; Chung K.	Athens	GA		

US-CL-CURRENT: 536/26.22; 536/27.14, 536/28.2

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KMOC
Draw Desc	Image									

☐ 16. Document ID: US 5411947 A

L2: Entry 16 of 17

File: USPT

May 2, 1995

US-PAT-NO: 5411947

DOCUMENT-IDENTIFIER: US 5411947 A

TITLE: Method of converting a drug to an orally available form by covalently bonding a lipid to the drug

DATE-ISSUED: May 2, 1995

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hostetler; Karl Y.	Del Mar	CA		
Kumar; Raj	San Diego	CA		

US-CL-CURRENT: 514/43; 514/12, 514/18, 514/192, 514/199, 514/2, 514/200, 514/50, 514/885

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KMOC
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☐ 17. Document ID: AU 200148301 A WO 200176555 A2

L2: Entry 17 of 17

File: DWPI

Oct 23, 2001

DERWENT-ACC-NO: 2001-663003

DERWENT-WEEK: 200213

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TITLE: New lipid-based drug delivery system for the treatment of diseases including localized increase in specific extracellular phospholipases activity, comprises an

active drug such as lysolipid derivative

INVENTOR: DAVIDSEN, J; FROKJAER, S ; JORGENSEN, K ; MOURITSEN, O G ; VERMEHREN, C

PRIORITY-DATA: 2000DK-0000616 (April 12, 2000)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
AU 200148301 A	October 23, 2001		000	A61K009/127
WO 200176555 A2	October 18, 2001	E	066	A61K009/127

INT-CL (IPC): A61 K 7/00; A61 K 9/127; A61 P 17/00

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KM/C
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L2: Entry 5 of 17

File: USPT

Dec 26, 2000

DOCUMENT-IDENTIFIER: US 6166089 A

TITLE: Prodrugs with enhanced penetration into cells

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temporally correlated to alterations in myocytic anaerobic metabolism and electron microscopic analyses.

Other Reference Publication (8):

Natarajan et al. "Activation of endothelial cell phospholipase D by hydrogen peroxide and fatty acid hydroperoxide." J. Biol. Chem 268(2):930-7.

Other Reference Publication (9):

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CLAIMS:

9. The method according to claim 8, wherein said lipase is phospholipase.

10. The method according to claim 9, wherein said phospholipase is phospholipase A.sub.2.

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L2: Entry 15 of 17

File: USPT

Jan 16, 1996

DOCUMENT-IDENTIFIER: US 5484911 A

TITLE: Nucleoside 5'-diphosphate conjugates of ether lipids

Brief Summary Text (9):

Previously, anti-HIV nucleoside conjugates of phospholipids (diacyl, ether thioether, and amido) have been reported. Dimyristoyl-phosphatidylAZT, dimyristoylphosphatidyl-ddT, dimyristoylphosphatidyl-ddC, and AZT dipalmitoylglycerol were encapsulated in liposomes and these liposomal liponucleotides were shown to have antiretroviral activity in HIV-infected U937 (IC<sub>50</sub> 0.2-12  $\mu$ M) and CEM cells (IC<sub>50</sub> 1.7-22  $\mu$ M) (Hostettler, K. Y. et al., J. Biol. Chem. 265, 6112, 1990). AZT and ddI conjugates of amidoalkyl, oxyalkyl, and thioalkyl ether lipids through phosphate and phosphonate linkages showed promising in vitro activity (IC<sub>50</sub> 0.02-1.56  $\mu$ M) with 5-10 fold reduction in cell cytotoxicity compared to AZT alone (Piantadosi, C. et al., J. Med. Chem. 34, 1408, 1991). Hostettler's conjugates are those with non-antiretroviral diacylglycerides with phosphodiester or pyrophosphate diester bond. Thus, these conjugates are considered to be simple prodrugs of anti-HIV nucleosides. Since the majority are conjugates with a phosphodiester (monophosphate) bond, they cannot form micelles by sonication; therefore, they must be encapsulated in liposomes for testing. Also, Piantadosi's conjugates contain monophosphate or phosphonate bonds which cannot be formulated in micellar solution by sonication.

Brief Summary Text (15):

As illustrated in FIG. 1, the intact conjugate may work as a biotransformed prodrug with favorable properties such as sustained release of the nucleoside, resistance to hydrolysis by catabolic enzymes, and target specific delivery--selectivity. The conjugate may be metabolized in the cells to the nucleoside 5'-monophosphate and the glycerol or its 3'-phosphate. The 5'-nucleotide may be further phosphorylated to the nucleoside 5'-triphosphate, which inhibits the viral reverse transcriptase. The phosphatidic acid may be metabolized to 1-O-(or S)-alkyl-2-O-acylglycerol (or 1-thioglycerol) by phosphohydrolase and the latter may serve as a substrate for cholinephosphotransferase (EC 2.7.8.2) to produce 1-O-(or S)-alkyl-2-acylphosphatidylcholine. This might be then deacylated to the corresponding lysophosphatidyl-choline via a phospholipase A<sub>2</sub> reaction and release the fatty acid such as 13-oxamyristic acid or polyunsaturated fatty acid. The lysophosphocholine analog works both as an anti-HIV agent and a substrate of alkyl cleavage enzyme (tetrahydropteridine-dependent alkyl monooxygenase) releasing antiviral 1-docosanol or other long-chain alcohol.

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L2: Entry 16 of 17

File: USPT

May 2, 1995

DOCUMENT-IDENTIFIER: US 5411947 A

TITLE: Method of converting a drug to an orally available form by covalently bonding a lipid to the drug

Parent Case Text (1):

This application is a continuation-in-part of the following U.S. applications: Ser. No. 07/373,088, filed Jun. 28, 1989, and titled "Lipid Derivatives of Antiviral Nucleosides", now U.S. Pat. No. 5,223,263; Ser. No. 07/440,898, filed Nov. 22, 1989, and titled "Lipid Derivatives of Phosphonoacids for Liposome Incorporation and Method of Use", now U.S. Pat. No. 5,194,654; and Ser. No. 07/932,231, filed Aug. 19, 1992 now abandoned, and titled "Lipid Prodrugs of Salicylate and other Non-Steroidal Anti-Inflammatory Drugs." These applications are hereby incorporated by reference in their entirety.

Brief Summary Text (4):

Under normal circumstances, intact dietary lipids, mostly triglycerides and diglyceride phospholipids, are not readily absorbed through the intestinal mucosa. Phospholipids are present physiologically in the gut as phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidylglycerol, and phosphatidic acid. The normal physiological mechanism for lipid absorption requires conversion of the phosphatidyl forms of phospholipid to lysophospholipids, by removal of the sn-2 acyl group by the hydrolytic action of the pancreatic enzyme phospholipase A.sub.2 on the sn-2 acyl ester bond. Conversion of lipids to phospholipids and then to lysophospholipids thus provides the normal mechanism for absorption and transport of lipids from the gut, and accounts for the uptake of several grams of phospholipid per day.



**WEST**

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L2: Entry 16 of 17

File: USPT

May 2, 1995

US-PAT-NO: 5411947

DOCUMENT-IDENTIFIER: US 5411947 A

TITLE: Method of converting a drug to an orally available form by covalently bonding a lipid to the drug

DATE-ISSUED: May 2, 1995

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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US-CL-CURRENT: 514/43; 514/12, 514/18, 514/192, 514/199, 514/2, 514/200, 514/50, 514/885

## CLAIMS:

What is claimed is:

1. A method of converting a drug that is unavailable or poorly available in a mammal through the oral route of administration to an orally available form, comprising:

(a) covalently linking a lipid species selected from the group consisting of 1-O-alkyl-sn-glycerol-3-phosphates, and 1-O-acyl-sn-glycerol-3-phosphates to a functional group of said drug either directly through the phosphate group of the lipid species or through a multifunctional linker molecule to form a lipid derivative of the drug;

(b) recovering the lipid derivative of the drug from the linking reaction mixture of step (a); and

(c) incorporating the lipid derivative of the drug into a therapeutic formulation suitable for oral administration.

2. The method of claim 1 wherein said lipid derivative is a 1-O-alkyl-sn-glycerol-3-phosphate derivative of a drug, of the formula ##STR4## wherein R is a substituted or unsubstituted, saturated or unsaturated, straight or branched chain, or cyclic C.sub.1-24 alkyl group, covalently linked to the glycerol moiety in an ether linkage;

n=0 to 2;

L is a linking molecule, of the formula X--(CH.sub.2).sub.n --Y

wherein X and Y are functional groups independently selected from hydroxyl, sulfhydryl, carboxyl and amine groups, and n=1 to 24; or

L is absent; and

D is a drug having a functional group selected from the group consisting of hydroxyl, sulfhydryl, carboxyl, or amino groups.

3. The method of claim 2, wherein the drug is an anticancer nucleoside having a carboxyl, hydroxyl, or amino group available for covalent binding.
4. The method of claim 3, wherein the drug is 9-.beta.-D-arabinofuranosylcytosine (ara-C), 5-fluorouridine, 6-mercaptopurine riboside, 9-.beta.-D-arabinofuranosyladenine (ara-A), or 2'-arafluoro-2-chloro-deoxyadenosine.
5. The method of claim 2, wherein the drug is 5-amino-4-imidazole carboxamide ribonucleoside (AICA-riboside).
6. The method of claim 2, wherein D is a therapeutic peptide, or a peptidomimetic of from 3 to 35 amino acid residues or analogues thereof.
7. The method of claim 6, wherein the drug is n-muramyl tripeptide.
8. The method of claim 6, wherein the drug is enalkiren.
9. The method of claim 2, wherein the drug is selected from the group consisting of antibiotics of the penicillin and cephalosporin class.
10. The method of claim 9, wherein the drug is selected from the group consisting of penicillin G, cefazolin, ceftazidime, ceftriaxone, piperacillin.
11. The method of claim 1, wherein said drug is 3'-azido-3'-deoxythymidine (AZT).
12. The method of claim 1, wherein said drug is 3'-azido-3'-acycloguanosine (ACG).

**WEST****End of Result Set**

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L3: Entry 1' of 1

File: USPT

May 8, 2001

DOCUMENT-IDENTIFIER: US 6229002 B1

TITLE: Platelet derived growth factor (PDGF) nucleic acid ligand complexes

Brief Summary Text (20):

A few instances have been reported where researchers have attached antisense oligonucleotides to Lipophilic Compounds or Non-Immunogenic, High Molecular Weight Compounds. Antisense oligonucleotides, however, are only effective as intracellular agents. Antisense oligodeoxyribonucleotides targeted to the epidermal growth factor (EGF) receptor have been encapsulated into Liposomes linked to folate via a polyethylene glycol spacer (folate-PEG-Liposomes) and delivered into cultured KB cells via folate receptor-mediated endocytosis (Wang et al. (1995) Proc. Natl. Acad. Sci. USA 92:3318-3322). In addition, alkylene diols have been attached to oligonucleotides (Weiss et al., U.S. Pat. No. 5,245,022). Furthermore, a Lipophilic Compound covalently attached to an antisense oligonucleotide has been demonstrated in the literature (EP 462 145 B1).

Brief Summary Text (23):

Platelet-derived growth factor (PDGF) was originally isolated from platelet lysates and identified as the major growth-promoting activity present in serum but not in plasma. Two homologous PDGF isoforms have been identified, PDGF A and B, which are encoded by separate genes (on chromosomes 7 and 22). The most abundant species from platelets is the AB heterodimer, although all three possible dimers (AA, AB and BB) occur naturally. Following translation, PDGF dimers are processed into approximately 30 kDa secreted proteins. Two cell surface proteins that bind PDGF with high affinity have been identified,  $\alpha$  and  $\beta$ . (Heldin et al. (1981) Proc. Natl. Acad. Sci., 78: 3664; Williams et al. (1981) Proc. Natl. Acad. Sci., 79: 5867). Both species contain five immunoglobulin-like extracellular domains, a single transmembrane domain and an intracellular tyrosine kinase domain separated by a kinase insert domain. The functional high affinity receptor is a dimer and engagement of the extracellular domain of the receptor by PDGF results in cross-phosphorylation (one receptor tyrosine kinase phosphorylates the other in the dimer) of several tyrosine residues. Receptor phosphorylation leads to a cascade of events that results in the transduction of the mitogenic or chemotactic signal to the nucleus. For example, in the intracellular domain of the PDGF B receptor, nine tyrosine residues have been identified that when phosphorylated interact with different src-homology 2 (SH2) domain-containing proteins including phospholipase C-g, phosphatidylinositol 3'-kinase, GTPase-activating protein and several adapter molecules like Shc, Grb2 and Nck (Heldin (1995) Cell 80: 213). In the last several years, the specificities of the three PDGF isoforms for the three receptor dimers ( $\alpha$ - $\alpha$ ,  $\alpha$ - $\beta$ , and  $\beta$ - $\beta$ ) has been elucidated. The  $\alpha$ -receptor homodimer binds all three PDGF isoforms with high affinity, the  $\beta$ -receptor homodimer binds only PDGF BB with high affinity and PDGF AB with approximately 10-fold lower affinity, and the  $\alpha$ - $\beta$ -receptor heterodimer binds PDGF BB and PDGF AB with high affinity (Westermarck & Heldin (1993) Acta Oncologica 32:101). The specificity pattern results from the ability of the A-chain to bind only to the  $\alpha$ -receptor and of the B-chain to bind to both  $\alpha$  and  $\beta$ -receptor subunits with high affinity.

Brief Summary Text (47):

In embodiments of the invention directed to Complexes comprised of a PDGF Nucleic Acid Ligand and a Non-Immunogenic, High Molecular Weight Compound, it is preferred that the Non-Immunogenic, High Molecular Weight Compound is Polyalkylene Glycol, more

preferably, polyethylene glycol (PEG). More preferably, the PEG has a molecular weight of about 10-80K. Most preferably, the PEG has a molecular weight of about 20-45K. In embodiments of the invention directed to Complexes comprised of a PDGF Nucleic Acid Ligand and a Lipophilic Compound, it is preferred that the Lipophilic Compound is a glycerolipid. In the preferred embodiments of the invention, the Lipid Construct is preferably a Lipid Bilayer Vesicle and most preferably a Liposome. In the preferred embodiment, the PDGF Nucleic Acid Ligand is identified according to the SELEX method.

Drawing Description Text (9):

FIGS. 8A-8B show the substitution pattern compatible with high affinity binding to PDGF-AB. In FIGS. 8A-8C, the underlined symbols indicate 2'-O-methyl-2'-deoxynucleotides; italicized symbols indicate 2'-fluoro-2'-deoxynucleotides; normal font indicates 2'-deoxyribonucleotides; [3'T] indicates inverted orientation (3'3') thymidine nucleotide (Glen Research, Sterling, Va.); PEG in the loops of helices II and III of FIG. 8B indicates pentaethylene glycol spacer phosphoramidite (Glen Research, Sterling, Va.) (See FIG. 9 for molecular description). FIG. 8C shows the predicted secondary structure of a scrambled Nucleic Acid Ligand sequence that was used as a control in Examples 8 and 9. The scrambled region is boxed to accent the overall similarity of the scrambled Nucleic Acid Ligand to the Nucleic Acid Ligand shown in FIG. 8B.

Drawing Description Text (10):

FIGS. 9A-9E show the molecular descriptions NX31975 40K PEG (FIG. 9A), NX31976 40K (FIG. 9B), hexaethylene glycol phosphoramidite (FIG. 9C), pentyl amino linker (FIG. 9D), and 40K PEG NHS ester (FIG. 9E). The 5' phosphate group shown in the PEG Spacer of FIGS. 9A and 9B are from the hexaethylene glycol phosphoramidite.

Drawing Description Text (12):

FIG. 11 shows that NX31975-40K PEG significantly inhibited ( $p < 0.05$ ) about 50% of the neointima formation in rats based on the intima/media ratio for the control (PBS) and NX31975-40K PEG groups.

Drawing Description Text (13):

FIG. 12 shows the effects of NX31975 40K PEG on mitogen-stimulated proliferation of mesangial cells in culture (all mitogens were added at 100 ng/ml final concentration). Scrambled Nucleic Acid Ligand NX31976 and 40K PEG were also tested. Data are optical densities measured in the XTT assay and are expressed as percentages of baseline, i.e., cells stimulated with medium plus 200  $\mu\text{g/ml}$  40K PEG (i.e., the amount equivalent to the PEG attached to 50  $\mu\text{g/ml}$  Nucleic Acid Ligand). Results are means  $\pm$  SD of 5 separate experiments ( $n=3$  in the case of medium plus 40K PEG; statistical evaluation was therefore confined to NX31975 and scrambled Nucleic Acid Ligand groups).

Drawing Description Text (14):

FIGS. 13A-13E show effects of NX31975 40K PEG on glomerular cell proliferation (FIG. 13A), expression of glomerular PDGF B-chain (FIG. 13B), proteinuria in rats with anti-Thy 1.1 nephritis (FIG. 13C), mesangial cell activation (as assessed by glomerular de novo expression of  $\alpha$ -smooth muscle actin) (FIG. 13D), and monocyte/macrophage influx (FIG. 13E). NX31975 40K PEG is shown as black, NX31976 40K PEG is shown as cross-hatched, 40K PEG is shown as white, PBS is shown as hatched, and the normal range is shown as stippled.

Drawing Description Text (15):

FIGS. 14A-C shows the effects of NX31975 40K PEG on glomerular matrix accumulation. Glomerular immunostaining scores for fibronectin and type IV collagen as well as glomerular scores for type IV collagen mRNA expression (in situ hybridization) are shown. NX31975 40K PEG is shown as black, NX31976 40K PEG is shown as cross-hatched, 40K PEG is shown as white, PBS is shown as hatched, and the normal range is shown as stippled.

Detailed Description Text (21):

"Linker" is a molecular entity that connects two or more molecular entities through Covalent Bond or Non-Covalent Interactions, and can allow spatial separation of the molecular entities in a manner that preserves the functional properties of one or

more of the molecular entities. A linker can also be known as a Spacer. Examples of Linkers, include but are not limited to, the structures shown in FIGS. 9C-9E and the PEG spacer shown in FIG. 9A.

Detailed Description Text (30):

In another embodiment, the Complex of the present invention is comprised of a PDGF Nucleic Acid Ligand covalently attached to a Lipophilic Compound, such as a glycerolipid, or a Non-Immunogenic, High Molecular Weight Compound, such as Polyalkylene Glycol or polyethylene glycol (PEG). In these cases, the pharmacokinetic properties of the Complex will be enhanced relative to the PDGF Nucleic Acid Ligand alone. In another embodiment, the pharmacokinetic properties of the PDGF Nucleic Acid Ligand is enhanced relative to the PDGF Nucleic Acid Ligand alone when the PDGF Nucleic Acid Ligand is covalently attached to a Non-Immunogenic, High Molecular Weight Compound or Lipophilic Compound and is further associated with a Lipid Construct or the PDGF Nucleic Acid Ligand is encapsulated within a Lipid Construct.

Detailed Description Text (43):

It is to be understood that the therapeutic or diagnostic agent can also be associated with the surface of the Lipid Bilayer Vesicle. For example, a drug can be attached to a phospholipid or glyceride (a prodrug). The phospholipid or glyceride portion of the prodrug can be incorporated into the lipid bilayer of the Liposome by inclusion in the lipid formulation or loading into preformed Liposomes (see U.S. Pat. Nos. 5,194,654 and 5,223,263, which are incorporated by reference herein).

Detailed Description Text (45):

Lee and Low (1994, JBC 269:3198-3204) and DeFrees et al. (1996, JACS 118:6101-6104) first showed that co-formulation of ligand-PEG-lipid with lipid components gave liposomes with both inward and outward facing orientations of the PEG-ligand. Passive anchoring was outlined by Zalipsky et al. (1997, Bioconj. Chem. 8:111-118) as a method for anchoring oligopeptide and oligosaccharide ligands exclusively to the external surface of liposomes. The central concept presented in their work is that oligo-PEG-lipid conjugates can be prepared and then formulated into pre-formed liposomes via spontaneous incorporation ("anchoring") of the lipid tail into the existing lipid bilayer. The lipid group undergoes this insertion in order to reach a lower free energy state via the removal of its hydrophobic lipid anchor from aqueous solution and its subsequent positioning in the hydrophobic lipid bilayer. The key advantage to such a system is that the oligo-lipid is anchored exclusively to the exterior of the lipid bilayer. Thus, no oligo-lipids are wasted by being unavailable for interactions with their biological targets by being in an inward-facing orientation.

Detailed Description Text (46):

The efficiency of delivery of a PDGF Nucleic Acid Ligand to cells may be optimized by using lipid formulations and conditions known to enhance fusion of Liposomes with cellular membranes. For example, certain negatively charged lipids such as phosphatidylglycerol and phosphatidylserine promote fusion, especially in the presence of other fusogens (e.g., multivalent cations like Ca<sup>2+</sup>, free fatty acids, viral fusion proteins, short chain PEG, lysolecithin, detergents and surfactants). Phosphatidylethanolamine may also be included in the Liposome formulation to increase membrane fusion and, concomitantly, enhance cellular delivery. In addition, free fatty acids and derivatives thereof, containing, for example, carboxylate moieties, may be used to prepare pH-sensitive Liposomes which are negatively charged at higher pH and neutral or protonated at lower pH. Such pH-sensitive Liposomes are known to possess a greater tendency to fuse.

Detailed Description Text (67):

It is further contemplated by this invention that the agent to be delivered can be incorporated into the Complex in such a way as to be associated with the outside surface of the Liposome (e.g., a prodrug, receptor antagonist, or radioactive substance for treatment or imaging). As with the PDGF Nucleic Acid Ligand, the agent can be associated through covalent or Non-Covalent Interactions. The Liposome would provide targeted delivery of the agent extracellularly, with the Liposome serving as a Linker.

Detailed Description Text (68):

In another embodiment, a Non-Immunogenic, High Molecular Weight Compound (e.g., PEG) can be attached to the Liposome to provide Improved Pharmacokinetic Properties for the Complex. PDGF Nucleic Acid Ligands may be attached to the Liposome membrane or may be attached to a Non-Immunogenic, High Molecular Weight Compound which in turn is attached to the membrane. In this way, the Complex may be shielded from blood proteins and thus be made to circulate for extended periods of time while the PDGF Nucleic Acid Ligand is still sufficiently exposed to make contact with and bind to its Target.

Detailed Description Text (73):

In another embodiment of the invention, the Lipid Construct comprises a PDGF Nucleic Acid Ligand or Complex. In this embodiment, the glycerolipid can assist in the incorporation of the PDGF Nucleic Acid Ligand into the Liposome due to the propensity for a glycerolipid to associate with other Lipophilic Compounds. The glycerolipid in association with a PDGF Nucleic Acid Ligand can be incorporated into the lipid bilayer of the Liposome by inclusion in the formulation or by loading into preformed Liposomes. The glycerolipid can associate with the membrane of the Liposome in such a way so as the PDGF Nucleic Acid Ligand is projecting into or out of the Liposome. In embodiments where the PDGF Nucleic Acid Ligand is projecting out of the Complex, the PDGF Nucleic Acid Ligand can serve in a targeting capacity. It is to be understood that additional compounds can be associated with the Lipid Construct to further improve the Pharmacokinetic Properties of the Lipid Construct. For example, a PEG may be attached to the exterior-facing part of the membrane of the Lipid Construct.

Detailed Description Text (74):

In other embodiments, the Complex of the present invention is comprised of a PDGF Nucleic Acid Ligand covalently linked to a Non-Immunogenic, High Molecular Weight Compound such as Polyalkylene Glycol or PEG. In this embodiment, the pharmacokinetic properties of the Complex are improved relative to the PDGF Nucleic Acid Ligand alone. The Polyalkylene Glycol or PEG may be covalently bound to a variety of positions on the PDGF Nucleic Acid Ligand. In embodiments where Polyalkylene Glycol or PEG are used, it is preferred that the PDGF Nucleic Acid Ligand is bonded through the 5' hydroxyl group via a phosphodiester linkage.

Detailed Description Text (75):

In certain embodiments, a plurality of Nucleic Acid Ligands can be associated with a single Non-Immunogenic, High Molecular Weight Compound, such as Polyalkylene Glycol or PEG, or a Lipophilic Compound, such as a glycerolipid. The Nucleic Acid Ligands can all be to PDGF or PDGF and a different Target. In embodiments where there are multiple PDGF Nucleic Acid Ligands, there is an increase in avidity due to multiple binding interactions with PDGF. In yet further embodiments, a plurality of Polyalkylene Glycol, PEG, glycerol lipid molecules can be attached to each other. In these embodiments, one or more PDGF-Nucleic Acid Ligands or Nucleic Acid Ligands to PDGF and other Targets can be associated with each Polyalkylene Glycol, PEG, or glycerol lipid. This also results in an increase in avidity of each Nucleic Acid Ligand to its Target. In embodiments where multiple PDGF Nucleic Acid Ligands are attached to Polyalkylene Glycol, PEG, or glycerol lipid, there is the possibility of bringing PDGF molecules in close proximity to each other in order to generate specific interactions between PDGF. Where multiple Nucleic Acid Ligands specific for PDGF and different Targets are attached to Polyalkylene Glycol, PEG, or glycerol lipid, there is the possibility of bringing PDGF and another Target in close proximity to each other in order to generate specific interactions between the PDGF and the other Target. In addition, in embodiments where there are Nucleic Acid Ligands to PDGF or Nucleic Acid Ligands to PDGF and different Targets associated with Polyalkylene Glycol, PEG, or glycerol lipid, a drug can also be associated with Polyalkylene Glycol, PEG, or glycerol lipid. Thus the Complex would provide targeted delivery of the drug, with Polyalkylene Glycol, PEG, or glycerol lipid serving as a Linker.

Detailed Description Text (88):

The following examples are provided to explain and illustrate the present invention and are not to be taken as limiting of the invention. Example 1 describes the various materials and experimental procedures used in Examples 2-4 for the generation of ssDNA ligands to PDGF and tests associated therewith. Example 2 describes the ssDNA ligands to PDGF and the predicted secondary structure of selected nucleic acid

ligands and a shared secondary structure motif. Example 3 describes the minimum sequence necessary for high affinity binding, the sites on the nucleic acid ligands and PDGF that are in contact, inhibition by DNA ligands of PDGF isoforms on cultured cells, and inhibition of mitogenic effects of PDGF in cells by DNA ligands. Example 4 describes substitutions of SELEX-derived ligands with modified nucleotides. Example 5 describes synthesis of PEG-modified PDGF Nucleic Acid Ligands. Example 6 describes stability of modified ligands in serum. Example 7 describes efficacy of a modified ligand (NX31975-40K PEG) in restenosis. Example 8 describes the various materials and method used in Example 9 for testing the inhibition of PDGF in glomerulonephritis. Example 9 describes inhibition of PDGF in glomerulonephritis. Example 10 describes the experimental procedures for evolving 2'-fluoro-2'-deoxypyrimidine RNA ligands to PDGF and the RNA sequences obtained.

Detailed Description Text (133):

SYNTHESIS OF PEG-MODIFIED PDGF NUCLEIC ACID LIGANDS

Detailed Description Text (137):

B) Conjugation of 40K PEG NHS ester to the aminolinker on PDGF Nucleic Acid Ligands

Detailed Description Text (138):

The NX31975 crude oligonucleotide containing the 5' primary amino group was dissolved in 100 mM sodium borate buffer (pH 9) to 60 mg /ml concentration. In a separate tube 2 Eq of PEG NHS ester (FIG. 9E) (Shearwater Polymers, Inc.) was dissolved in dry DMF (Ratio of borate: DMF 1:1) and the mixture was warmed to dissolve the PEG NHS ester. Then the oligo solution was quickly added to PEG solution and the mixture was vigorously stirred at room temperature for 10 minutes. About 95% of the oligo conjugated to the PEG NHS ester.

Detailed Description Text (143):

EFFICACY OF NX31975-40K PEG IN RESTENOSIS

Detailed Description Text (144):

Rat Restenosis Model and Efficacy Results. The plasma residence time of Nucleic Acid Ligands is dramatically improved by the addition of large, inert functional groups such as polyethylene glycol (see for example PCT/US 97/18944). For in vivo efficacy experiments, 40K PEG was conjugated to NX31975 to create NX31975 40K PEG as described in Example 5B (see FIG. 9A for molecular description). Importantly, based on binding experiments, the addition of 40 kDa PEG group at the 5'-end of the ligand does not affect its binding affinity for PDGF-BB.

Detailed Description Text (145):

The effect of selective inhibition of PDGF-B by NX31975-40K PEG was studied in three-month-old male Sprague-Dawley rats (370-450 g). The rats were housed three to a cage with free access to a standard laboratory diet and water. Artificial light was provided 14 hours per day. The experiments were performed in accordance with the institutional guidelines at the Animal Department, Department of Surgery, University Hospital, Uppsala University, Sweden.

Detailed Description Text (146):

A total of 30 rats were randomly allocated to one of two treatment groups: 15 rats in group one received 10 mg/kg body weight of NX3 1975-40K PEG in phosphate buffered saline (PBS) twice daily delivered by intraperitoneal (i.p.) injections and 15 rats in group two (the control group) received an equal volume of PBS (about 1 ml). The duration of treatment was 14 days. The first injections in both groups were given one hour before arterial injury.

Detailed Description Text (152):

ANTAGONISM OF PDGF IN GLOMERULONEPHRITIS BY NX31975-40K PEG

Detailed Description Text (155):

All Nucleic Acid Ligands and their sequence-scrambled controls were synthesized by the solid phase phosphoramidite method on controlled pore glass using an 8800 Milligen DNA Synthesizer and deprotected using ammonium hydroxide at 55.degree. C. for 16 h. The Nucleic Acid Ligand used in experiments described in this example and Example 9 is NX31975 40K PEG (FIG. 9A). NX31975 40K PEG was created by conjugating

NX31975 (Table 7) to 40K PEG as described in Example 5. In the sequence-scrambled control Nucleic Acid Ligand, eight nucleotides in the helix junction region of NX31975 were interchanged without formally changing the consensus secondary structure (see FIG. 8C). The binding affinity of the sequence-scrambled control Nucleic Acid Ligand for PDGF BB is about  $1 \mu\text{M}$ , which is 10,000 fold lower compared to NX21617. The sequence-scrambled control Nucleic Acid Ligand was then conjugated to PEG and named NX31976 40K PEG (see FIG. 9B for molecular description). The covalent coupling of PEG to the Nucleic Acid Ligand (or to the sequence-scrambled control) was accomplished as described in Example 5.

Detailed Description Text (158):

Human mesangial cells were established in culture, characterized and maintained as described previously (Radeke et al. (1994) J. Immunol. 153:1281-1292). To examine the antiproliferative effect of the ligands on the cultured mesangial cells, cells were seeded in 96-well plates (Nunc, Wiesbaden, Germany) and grown to subconfluency. They were then growth-arrested for 48 hours in MCDB 302 medium (Sigma, Deisenhofen, Germany). After 48 hours various stimuli together with either 50 or 10  $\mu\text{g/ml}$  Nucleic Acid Ligand NX31975 40K PEG or 50 or 10  $\mu\text{g/ml}$  sequence-scrambled Nucleic Acid Ligand (NX31976 40K PEG) were added: medium alone, 100 ng/ml human recombinant PDGF-AA, -AB or -BB (kindly provided by J. Hoppe, University of Wurzburg, Germany), 100 ng/ml human recombinant epidermal growth factor (EGF; Calbiochem, Bad Soden, Germany) or 100 ng/ml recombinant human fibroblast growth factor-2 (kindly provided by Synergen, Boulder, Colo.). Following 72 hours of incubation, numbers of viable cells were determined using 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide (XTT; Sigma) as described (Lonnemann et al. (1995) Kidney Int. 51:837-844).

Detailed Description Text (160):

Anti-Thy 1.1 mesangial proliferative glomerulonephritis was induced in 33 male Wistar rats (Charles River, Sulzfeld, Germany) weighing 150-160g by injection of 1 mg/kg monoclonal anti-Thy 1.1 antibody (clone OX-7; European Collection of Animal Cell Cultures, Salisbury, England). Rats were treated with Nucleic Acid Ligands or PEG (see below) from day 3 to 8 after disease induction. Treatment consisted of twice daily i.v. bolus injections of the substances dissolved in 400  $\mu\text{l}$  PBS, pH 7.4. The treatment duration was chosen to treat rats from about one day after the onset to the peak of mesangial cell proliferation (Floege et al. (1993) Kidney Int. Suppl. 39:S47-54). Four groups of rats were studied: 1) nine rats, who received NX31975 40K PEG (i.e., a total of 4 mg of the PDGF-B ligand coupled to 15.7 mg 40K PEG); 2) ten rats, who received an equivalent amount of PEG-coupled, scrambled Nucleic Acid Ligand (NX31976 40K PEG); 3) eight rats, who received an equivalent amount (15.7 mg) of 40K PEG alone; 4) six rats, who received 400  $\mu\text{l}$  bolus injections of PBS alone. Renal biopsies for histological evaluation were obtained on days 6 and 9 after disease induction. Twenty-four hour urine collections were performed from days 5 to 6 and 8 to 9 after disease induction. The thymidine analogue 5-bromo-2'-deoxyuridine (BrdU; Sigma, Deisenhofen, Germany; 100 mg/kg body weight) was injected intraperitoneally at 4 hours prior to sacrifice on day 9.

Detailed Description Text (176):

For all experiments reported here, the modified DNA Nucleic Acid Ligand was conjugated to 40K PEG as described in Examples 5 and 8 and shown in FIGS. 9A and 9B. Since most Nucleic Acid Ligands have molecular weights ranging between 8 to 12 kDa (the modified PDGF Nucleic Acid Ligand has MW of 10 kDa), the addition of a large inert molecular entity such as PEG dramatically improves the residence times of Nucleic Acid Ligands in vivo (see for example PCT/US 97/18944). Importantly, the addition of the PEG moiety to the 5' end of the Nucleic Acid Ligand has no effect on the binding affinity of the Nucleic Acid Ligand for PDGF-BB ( $K_{\text{sub.d.}} \text{ about } 1 \times 10^{-10} \text{ M}$ ).

Detailed Description Text (180):

In growth arrested mesangial cells, the effects of NX31975 40K PEG or the scrambled Nucleic Acid Ligand (NX31976 40K PEG) on growth factor induced proliferation were tested. Stimulated growth rates of the cells were not affected by the addition of scrambled Nucleic Acid Ligand (FIG. 12). Fifty  $\mu\text{g/ml}$  of NX31975 40K PEG significantly reduced PDGF-BB induced mesangial cell growth (FIG. 12). PDGF-AB and -AA induced mesangial cell growth also tended to be lower with NX31975 40K PEG, but



these differences failed to reach statistical significance (FIG. 12). In contrast, no effects of NX31975 40K PEG on either EGF or FGF-2 induced growth were noted. Similar effects were noted if the Nucleic Acid Ligands were used at a concentration of 10 .mu.g/ml (data not shown).

Detailed Description Text (182):

Following the injection of anti-Thy 1.1 antibody, PBS treated animals developed the typical course of the nephritis, which is characterized by early mesangiolysis and followed by a phase of mesangial cell proliferation and matrix accumulation on days 6 and 9 (Floege et al. (1993) Kidney Int. Suppl. 39: S47-54). No obvious adverse effects were noted following the repeated injection of Nucleic Acid Ligands or PEG alone, and all rats survived and appeared normal until the end of the study.

Detailed Description Text (183):

In PAS stained renal sections the mesangioproliferative changes on days 6 and 9 after disease induction were severe and indistinguishable among rats receiving PBS, PEG alone or the scrambled Nucleic Acid Ligand (data not shown). Histological changes were markedly reduced and almost normalized in the NX31975 40K PEG ligand treated group. In order to (semi-)quantitatively evaluate the mesangioproliferative changes, various parameters were analyzed:

Detailed Description Text (185):

Glomerular cell proliferation, as assessed by counting the number of glomerular mitoses, was not significantly different between the three control groups on days 6 and 9 (FIG. 13A). As compared to rats receiving the scrambled Nucleic Acid Ligand, treatment with PDGF-B ligand led to a reduction of glomerular mitoses by 64% on day 6 and by 78% on day 9 (FIG. 13A). To assess the treatment effects on mesangial cells, the renal sections for .alpha.-smooth muscle actin were immunostained, which is expressed by activated mesangial cells only (Johnson et al. (1991) J. Clin. Invest. 87:847-858). Again, there were no significant differences between the three control groups on days 6 and 9. However, the immuno staining scores of .alpha.-smooth muscle actin were significantly reduced on day 6 and 9 in the NX31975 40K PEG treated group (FIG. 13D). To specifically determine whether mesangial cell proliferation was reduced, NX31975 40K PEG treated rats and scrambled Nucleic Acid Ligand treated rats were double immunostained for a cell proliferation marker (BrdU) and .alpha.-smooth muscle actin. The data confirmed a marked decrease of proliferating mesangial cells on day 9 after disease induction: 2.2+-.0.8 BrdU/.alpha.-smooth muscle actin positive cells per glomerular cross section in PDGF-B aptamer treated rats versus 43.3+-.12.4 cells in rats receiving the scrambled Nucleic Acid Ligand, i.e., a 95% reduction of mesangial cell proliferation. In contrast, no effect of the PDGF-B aptamer was noted on proliferating monocytes/macrophages on day 9 after disease induction (PDGF-B aptamer treated rats: 2.8+-.1.1 BrdU+/ED-1+ cells per 100 glomerular cross sections; scrambled aptamer treated rats: 2.7+-.1.8).

Detailed Description Text (187):

By immunohistochemistry, the glomerular PDGF B-chain expression was markedly upregulated in all three control groups (FIG. 13B), similar to previous observations (Yoshimura et al. (1991) Kidney Int. 40:470-476). In the NX31975 40K PEG treated group the glomerular overexpression of PDGF B-chain was significantly reduced in parallel with the reduction of proliferating mesangial cells (FIG. 13B).

Detailed Description Text (189):

The glomerular monocyte/macrophage influx was significantly reduced in the NX31975 40K PEG treated rats as compared to rats receiving scrambled Nucleic Acid Ligand on days 6 and 9 after disease induction (FIG. 13E).

Detailed Description Text (191):

Moderate proteinuria of up to 147 mg/24 hrs was present on day 6 after disease induction in the 3 control groups (FIG. 13C). Treatment with NX31975 40K PEG reduced the mean proteinuria on day 6, but this failed to reach statistical significance (FIG. 13C). Proteinuria on day 9 after disease induction was low and similar in all four groups (FIG. 13C).

Detailed Description Text (193):

By immunohistochemistry, marked glomerular accumulation of type IV collagen and

fibronectin was noted in all three control groups (FIG. 14). The overexpression of both glomerular type IV collagen and fibronectin was significantly reduced NX31975 40K PEG treated rats (FIG. 14). In the latter, glomerular staining scores approached those observed in normal rats (FIG. 14). By in situ hybridization, the decreased glomerular expression of type IV collagen in NX31975 40K PEG treated rats was shown to be associated with decreased glomerular synthesis of this collagen type (FIG. 14).

**CLAIMS:**

11. The complex of claim 7 wherein said Complex is ##STR36##  
in which said PEG spacer is ##STR37##  
and said nucleic acid ligand comprises SEQ ID NO:146.

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L3: Entry 1 of 1

File: USPT

May 8, 2001

US-PAT-NO: 6229002

DOCUMENT-IDENTIFIER: US 6229002 B1

TITLE: Platelet derived growth factor (PDGF) nucleic acid ligand complexes

DATE-ISSUED: May 8, 2001

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Janjic; Nebojsa	Boulder	CO		
Gold; Larry	Boulder	CO		

US-CL-CURRENT: 536/23.1; 435/6, 435/91.2

Full	Title	Cl.	Rev.	CLS.	Ref.	Seq.	Att.
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L4: Entry 24 of 47

File: USPT

Nov 21, 2000

DOCUMENT-IDENTIFIER: US 6150407 A

TITLE: Methods for inhibiting angiogenesis

Detailed Description Text (67):

Monoclonal antibodies optionally conjugated to liposomes and directed against a tumor marker, TNF-.alpha., or a TNF-.alpha. receptor, is another strategy that can be employed. In addition, targeting of a marker on abnormal tumor vasculature can be employed. The targeting moiety when coupled to a toxic drug or radioisotope will act to concentrate the drug where it is needed. Ligands for tumor-associated vessel markers can also be used. For example, a cell adhesion molecule that binds to a tumor vascular element surface marker can be employed. Liposomes and other drug delivery systems can also be used, especially if their surface contains a ligand to direct the carrier preferentially to the tumor vasculature. Liposomes offer the added advantage of shielding the drug from most normal tissues, thereby reducing the inherent toxicity of many compounds. When coated with polyethylene glycol (PEG) (i.e., stealth liposomes) to minimize uptake by phagocytes and with a tumor vasculature-specific targeting moiety, liposomes offer longer plasma half-lives, lower non-target tissue toxicity, and increased efficacy over non-targeted drug. Other targeting strategies include, but are not limited to, ADEPT (antibody-directed enzyme prodrug therapy), GDEPT (gene-directed EPT) and VDEPT (virus-directed EPT). In ADEPT, the targeting of an inactive prodrug to a tumor mass is effected by an antibody against a tumor-associated marker. The enzyme milieu in or about the tumor transforms the prodrug into an active toxic agent that then acts on the tumor tissue. Similarly, differential gene expression or viral targeting at the tumor site is used to activate a prodrug into its active, toxic form in GDEPT and VDEPT, respectively. Other strategies include targeting differentially expressed genes, enzymes or surface markers that appear on tumor-associated vasculature, to effect control of tumor growth. Using the foregoing methods, the compounds of Formula I can be targeted to the tumor vasculature to effect control of tumor progression or to other sites of interest (e.g., endothelial cells).

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L4: Entry 36 of 47

File: USPT

Jan 12, 1999

DOCUMENT-IDENTIFIER: US 5859228 A

TITLE: Vascular endothelial growth factor (VEGF) nucleic acid ligand complexes

Brief Summary Text (21):

A few instances have been reported where researchers have attached antisense oligonucleotides to Lipophilic Compounds. Antisense oligonucleotides, however, are only effective as intracellular agents. Antisense oligodeoxyribonucleotides targeted to the epidermal growth factor (EGF) receptor have been encapsulated into Liposomes linked to folate via a polyethylene glycol spacer (folate-PEG-Liposomes) and delivered into cultured KB cells via folate receptor-mediated endocytosis (Wang et al. (1995) Proc. Natl. Acad. Sci. USA 92:3318-3322). In addition, a Lipophilic Compound covalently attached to an antisense oligonucleotide has been demonstrated in the literature (EP 462 145 B1).

Detailed Description Text (36):

It is to be understood that the therapeutic or diagnostic agent can also be associated with the surface of the Lipid Bilayer Vesicle. For example, a drug can be attached to a phospholipid or glyceride (a prodrug). The phospholipid or glyceride portion of the prodrug can be incorporated into the lipid bilayer of the Liposome by inclusion in the lipid formulation or loading into preformed Liposomes (see U.S. Pat. Nos. 5,194,654 and 5,223,263, which are incorporated by reference herein).

Detailed Description Text (38):

The efficiency of delivery of the VEGF Nucleic Acid Ligand to cells may be optimized by using lipid formulations and conditions known to enhance fusion of Liposomes with cellular membranes. For example, certain negatively charged lipids such as phosphatidylglycerol and phosphatidylserine promote fusion, especially in the presence of other fusogens (e.g., multivalent cations like  $\text{Ca}^{2+}$ , free fatty acids, viral fusion proteins, short chain PEG, lysolecithin, detergents and surfactants). Phosphatidylethanolamine may also be included in the Liposome formulation to increase membrane fusion and, concomitantly, enhance cellular delivery. In addition, free fatty acids and derivatives thereof, containing, for example, carboxylate moieties, may be used to prepare pH-sensitive Liposomes which are negatively charged at higher pH and neutral or protonated at lower pH. Such pH-sensitive Liposomes are known to possess a greater tendency to fuse.

Detailed Description Text (58):

It is further contemplated by this invention that the agent to be delivered can be incorporated into the Complex in such a way as to be associated with the outside surface of the Liposome (e.g., a prodrug, receptor antagonist, or radioactive substance for treatment or imaging). As with the VEGF Nucleic Acid Ligand, the agent can be associated through covalent or Non-Covalent Interactions. The Liposome would provide targeted delivery of the agent extracellularly, with the Liposome serving as a Linker.

## WEST Search History

DATE: Tuesday, August 27, 2002

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L4	L1 and peg	47	L4
L3	L2 and peg	1	L3
L2	L1 and phospholipase\$	17	L2
L1	prodrug\$ same liposome\$	177	L1

END OF SEARCH HISTORY